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NEW METHODS OF EARLY DIAGNOSIS OF INFECTIOUS DISEASES

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"Science advances in jolts, depending on the advances made in methodology. With each step of methodology forward we are, as it were, lifted a step higher, from which a wide horizon is opened up with subjects hitherto invisible" (I. P. Pavlov).

As is well known, the clinic of infectious diseases has not up to this time had at its disposal objective and convenient methods of studying the toxins and various liquids of the organism.

In 1941, in Trudakh pervoy sessii Moskovskogo obshchestva fiziologov, biokhimikov i farmakologov" [Transactions of the First Session of the Moscow Society of Physiologists, Biochemists, and Pharmacologists], Tarusov published a physicochemical method named by him the striction [striktsionnyy] method, with the aid of which the author succeeded in determining toxins in the blood and spinal fluid in horses which had been subjected to experimental infection with tetanus.

The simplicity of the striction (or toxinometric) method impelled us to test the possibility of its application among patients in the presence of natural infection with various agents of infectious diseases.

The striction method permits one to establish the presence of toxins in various biological liquids among infectious patients, the dynamics of intoxication, its connection with the stages of the diseases, the temperature, the severity of the state, etc.

The patterns obtainable in the presence of neutralization of toxins have also served as the basis for the use of the striction method with the object of early diagnosis of the most important infectious diseases. Further study of the method has permitted the sphere of its use to be broadened at the expense of infectious diseases in which field good prospects for its use for diagnostic purposes are also being opened up.

The method is based on the following principle: the reaction between a liquid and the molecules of live protein proceeds according to the laws of chemical kinetics and obeys definite quantitative patterns; under these conditions the molecules, into which the surviving tissue breaks down, swell; this leads to a diminution of the volume of water or to striction. The process of swelling of the cells of tissues placed in physiological solution appears to be a reflection of the state of their colloids (dispersion, solvation, i.e., capacity to absorb water) and is expressed as a smooth curve, which assumes the character of a parabola. Parallel to this the process of diminution of the water (at the expense of having linked with molecules which are formed in the presence of the break-down of protein), in other words, the curtailment of the volume, or the striction process, also proceeds just as smoothly as the process of swelling. Thus, the striction curve presents itself, as it were, as a mirror reflection of the swelling curve.

For observation of the process of swelling of the tissues both in a nontoxic, and in a toxic medium, a device is used consisting of a glass vessel 2-3 ml in capacity, with a capillary tube sealed in at the bottom which is bent up vertically upward, the height of which is equal approximately to 8-10 cm in the presence of a diameter of 0.1-0.2 mm.

The vessel is covered with a well-ground glass stopper. A second variant, which can be used for these purposes, presents just this difference, that the capillary tube goes off not from the vessel, but from the hollow stopper (Figure 1).

In practice the investigation is conducted in the following fashion. Into the vessel described, carefully washed with chromic mixture and distilled water, is introduced 0.8–0.9 g of the femoral muscle of the frog (or of another laboratory animal) preliminarily subjected to crushing [shattering] with a scissors. After this the vessel is filled up to the top with Ringer or physiological solution (at a pH = 7.2) which before the investigation is boiled (to remove bubbles of air) and is cooled. Then the vessel is tightly covered with a glass stopper, thickly smeared with a mixture of vaseline with paraffin, which is firmly attached to the vessel, having been tied to the neck of the vessel. At the same time the liquid is extruded into the capillary and fills it up. The charged vessel is immersed in a water thermostat in such a way that the capillary tube is raised above the level of the water. Excess water is also sucked off from the capillary with a piece of filter paper or wadding and after the appearance in it of a meniscus an observation is conducted through the reading device with an enlargement of 20–40 times. As the latter reading device the tube of a microscope horizontal microscope is used, fixed on a support, in which is placed a so-called ocular micrometer, or a ruler with divisions. The temperature of the water in the thermostat is kept strictly at a definite level in the limits of 25–30° for the course of the investigation, which lasts on the average 25–30 minutes (Figure 2) the reading of the level of the meniscus, gradually being lowered in the process of swelling of the protein, is conducted every 3 minutes, on the average 9-10 times; the indexes of the readings obtained, being transferred to millimeter paper along the ordinate axis (the time of the investigation is noted along the abscissa axis) permit one graphically to depict the curve of swelling of the tissue.

The swelling of colloids presents a hyperbolic function, being expressed by a smooth curve which assumes the character of a parabola. Parallel to this the process of diminution of water (at the expense of being bound with the molecules which are being formed in the presence of the breakdown of the protein), in other words, the curtailment of the volume, or the striction process, will also proceed just as smoothly as the process of swelling. Thus, the striction curve presents itself, as it were, as a mirror reflection of the curve of swelling and also assumes the character of a parabolic curve with a hyperbolic tendency (Figure 3).

The striction curves reflecting the normal course of swelling of surviving tissue can be distinguished by the steepness of the rise, but the general tendency remains identical. In their character the normal striction curves always approach the form of a parabolic curve. The addition to the solution even of insignificant traces of toxins changes the kinetics of the striction of tissues in the presence of their swelling, and as a result the character of the curve is also changed -- they become wave-like, as it were braked [inhibited] (Figure 4). The early appearance of "steps" and their degree of repetition argue in favor of a high concentration of toxin in the substrate being investigated.

We have conducted a considerable number of investigations (900), by which it was established that in the presence of the most important infectious diseases (typhus, virus grippe, meningitis, tetanus, zoonotic diseases, measles, scarlet fever, diphtheria, and others) the toxins at the height of the process are determined to the extent of 80-90%. The presence of toxins was also manifested in the blood in patients with pneumonia, tuberculosis of the lungs (in the period of exacerbations), with sepsis. In a number of cases the toxins were discovered in the blood of patients with noninfectious diseases, for example, in the presence of cancer, diseases of the blood, and others.

Investigation by the striction method of the spinal fluid in patients with meningitis (of various etiology) and with typhus fever with complications in the form of cases of meningitis and of meningo-encephalitis also permitted one to ascertain the presence of toxins.

The pathological striction curves which are obtainable in the investigation of biological liquids containing toxins are not distinguished by specificity and do not permit one to differentiate diverse infectious diseases. At the same time they provide the possibility of differentiating the degree of toxicity of the substrate being investigated in any period of the disease.

The study of the dynamics of the striction process, from the beginning of the disease up to the period of recovery, permits one to ascertain the disappearance of toxins in the biological liquids being investigated. In proportion to the improvement of the state of the patients the striction curves lose their pathological character, and in the period of convalescence, as a rule, they lose their complete normalization is noted.

We consider it possible to associate the normalization of the pathological curves with the action of the antitoxins which are detectable in the blood of convalescents. Proceeding from this, we mixed the blood of patients with typhoid fever and typhus with the blood (or serum) of individuals recovering from these same diseases. Such neutralized "mixtures" were placed for 1/2 to one hour in a thermostat, after which they were subjected to striction (or toxinometric) investigation. As a rule, the striction curves obtained in the investigation of liquids which had been subjected to neutralization had lost a pathological character. Thus, in the interests of the diagnosis, the substrate being investigated should be subjected to striction analysis before and after neutralization. This method permitted us in almost 80% of the patients to make an early diagnosis of such important diseases as typhoid fever and typhus (Table I).

TABLE I

NEUTRALIZATION ACCORDING TO DATA OF THE STRICTION OF THE BLOOD OF PATIENTS WITH TYPHOID FEVER AND WITH TYPHUS

Disease	Total Number of Cases	Neutralization Obtained	Neutralization not Obtained
Typhoid fever	109	84	25
Typhus	104	80 (77%)	24

In a number of cases the striction method was also applied with a positive result in later stages (second to third week) of cases of disease proceeding atypically or in an occult form.

Particularly clear-cut results in the sense of normalization of the striction curves were obtained in those cases in which the blood being investigated was neutralized with hyperimmune serum with a high titer (up to 1:40,000) obtained from rabbits immunized with typhoid fever trivaccine.

The comparative evaluation of the laboratory methods of diagnosis of typhoid fever and typhus most frequently being used in practice and the striction method (with neutralisation) worked out by us permits one to speak of the preferability of the striction method. The striction method permits one to diagnose the disease not only more often (Table 2), but also earlier.

TABLE 2

RESULTS OF THE DIAGNOSIS OF TYPHOID FEVER AND TYPHUS BY VARIOUS METHODS

Method of Diagnosis	Typhoid Fever		Typhus	
	Number of Patients	Positive Result (in %)	Number of Patients	Positive Result (in %)
Positive hemocultures	154	20	123	--
Agglutination Reaction in a diagnostic titer (second to third week of the disease)	154	50	123	62
Toxinometric [Striction method] analysis in the neutralisation variant (first week of the disease)	109	77	104	76.9

Cases of typhus in which the diagnosis was made on the first and second day of the disease, appear to be a demonstration of the possibility of using the striction method for early diagnosis. The subsequent course of the disease (the appearance of a petechial rash, a positive serological reaction, etc) completely confirmed our initial diagnosis.

Cross investigations of the blood of patients with typhoid fever and typhus, i.e., mixed blood of individuals sick with typhoid fever with the blood of typhus convalescents (and vice versa) did not lead to the normalization of the striction curves; mixing of the blood of those sick with the indicated diseases with the blood of healthy people or in general with any blood which was nontoxic (according to the striction signs) in exactly the same - did not cause the neutralization of the toxins and the striction curves of such mixtures proved to be pathological.

It was shown by us that the striction method can also be used in the presence of noninfectious diseases. The presence of toxins in the blood was noted among patients with cancer, in the presence of diseases of the blood, etc. At the same time, besides the possibility of studying the dynamics of toxicosis among noninfectious diseases, the striction method permits one to establish in vitro the detoxicating action of various biological and pharmacological agents. For this purpose we also resorted to preliminary neutralization which in the given case was of a nonspecific nature. The last variant -- nonspecific neutralization with subsequent striction analysis -- can be used for the diagnosis of noninfectious diseases. The observations of Brulakova and Stepanyan are a demonstration of this; they showed that the toxic blood of cancer patients was neutralized by heparin, at the same time that in control investigations (with the blood of patients with other diseases) this effect of neutralization was not manifested.

The data obtained permit one to consider that the striction method can be recommended for the early diagnosis of the most important infectious diseases. Its practical significance grows when another role which we assign to the struggle against intoxications in the treatment of infectious diseases is taken into account. In this connection it should be underlined that the striction method is devoid of those defects which are inherent in the most widely distributed biological method of detecting toxins -- the infection of animals: by virtue of the complexity and cumbersomeness of the latter it is not always accomplishable under the conditions of infectious [stationary] hospitals; besides that , it is well known that laboratory animals are sensitive only to certain toxins.

Conclusions

1. With the object of detecting toxins in the serum of blood (and also in the spinal fluid) of patients with infectious and non-infectious diseases a physicochemical method, the so-called striction method, can be used.

2. Normal striction curves in their character approximates a parabolic curve; in the presence of the addition to the solution of even insignificant quantities of toxins the character of the striction curves is changed; it becomes wave-like, which is evidence of the disturbance of the normal course of absorption of liquid.

3. In a study of the dynamics of the striction process the disappearance of toxins in the period of convalescence was established; this can be explained by the action of antitoxins appearing in the blood of convalescents.

4. With the object of making an early diagnosis of the most important infectious diseases (typhoid fever, typhus) the principle of neutralisation was used: the blood of the patients was mixed with the appropriate antitoxic serum or blood of a person who had been stricken with the disease which is assumed in the patient being investigated, and the diagnosis is made in those cases in which the pathological striction curve of the toxic blood is normalized (as a result of specific neutralization).

5. The early diagnosis of typhoid fever and typhus with the aid of the striction method was made in 77% of the cases, which exceeded the number of cases detected with the aid of the methods most

often being used in practice for the diagnosis of typhoid fever and typhus (the agglutination reaction, the isolation of hemocultures). This permits one to speak of the advantage of the striction method. Moreover the latter is devoid of the defects inherent in the most widespread biological method of detecting toxins -- the infection of animals -- which, as is well known, are sensitive only to definite toxins, while the striction method permits one to detect any bacterial toxin and to judge concerning its approximate concentration.

6. Besides use with the object of early diagnosis, the striction method can be applied for the evaluation of the results of the treatment of infectious diseases (on the basis of the lowering of the toxicity of the blood of a patient under the influence of a medicinal agent).

FIGURES

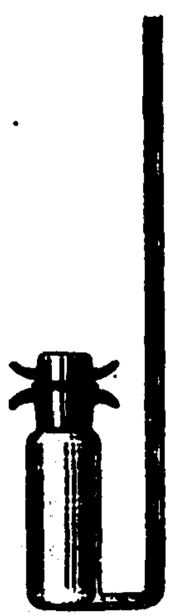

Figure 1

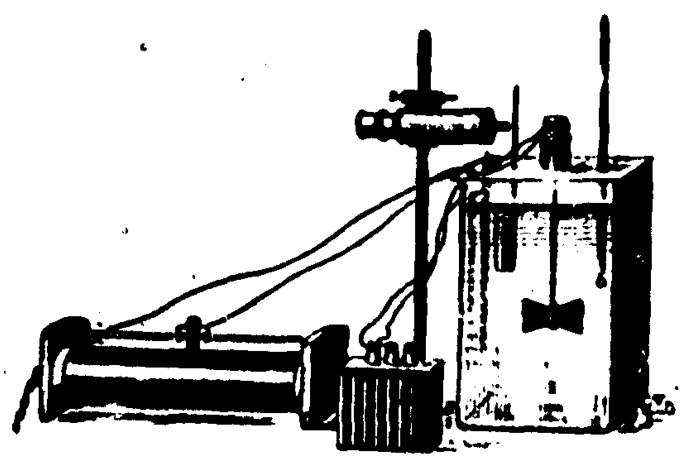

Figure 2

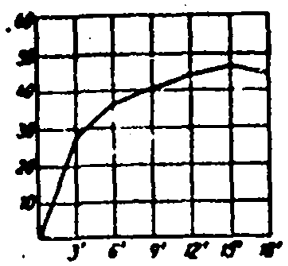

Figure 3

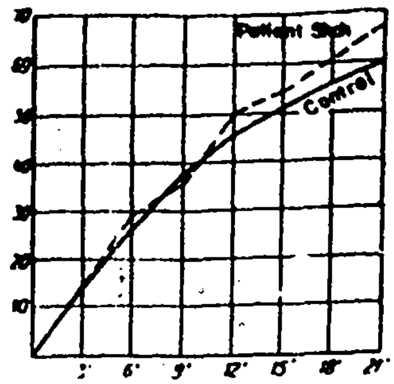

Figure 4